REMARKS

1. Status of claims

After entry of the above amendment, claims 1-23 and 129-130 are pending and under consideration.

2. Support for amendment

The above amendment clarifies language the Examiner alleged was indefinite. No new matter has been added by this amendment.

3. Claim rejections under 35 U.S.C. § 112, second paragraph

The Examiner rejected claims 14 and 16-17 as allegedly being indefinite. Specifically, he alleged the recitation of "the first culture medium" lacked antecedent basis. Applicants thank him for noticing this inadvertent error. The above amendment replaces this term with "the minimal medium," which has antecedent basis.

Therefore, Applicants request this rejection of claims 14 and 16-17 be withdrawn.

4. Claim rejections under 35 U.S.C. § 103(a)

The Examiner rejected claims 1-7, 12-20, 22-23, and 129-130 as allegedly being obvious over Rajgarhia, *et al.*, US 7,229,805 ("Rajgarhia") in view of Lee, *et al.*, UK 2251864 ("Lee"). Applicants traverse this rejection.

According to MPEP 2142, to reach a proper determination of obviousness under 35 U.S.C. §103(a), the examiner must step backward in time and into the shoes worn by the hypothetical "person of ordinary skill in the art" when the invention was unknown and just

before it was made. In view of all factual information, the examiner must then make a determination whether the claimed invention *"as a whole"* would have been obvious at that time to that person (emphasis added). Knowledge of applicant's disclosure must be put aside in reaching this determination, yet kept in mind in order to determine the "differences," conduct the search and evaluate the *"subject matter as a whole"* of the invention (emphasis added). The tendency to resort to "hindsight" based upon applicant"s disclosure is often difficult to avoid due to the very nature of the examination process. However, impermissible hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art.

Let us consider the person of ordinary skill in the art of yeast fermentation, interested in the production of lactic acid and having Rajgarhia before him, just before the present invention was made. He or she would note the following passages of Rajgarhia.

Col. 19, lines 6-27:

> The invention provides methods for producing organic products using any of the yeast cells or other microbial cells provided herein. Such methods involve providing yeast cells and culturing the provided yeast cells with culture medium such that an organic product (e.g., glycerol, acrylate, xylose, ascorbate, lactate, citrate, isocitrate, .alpha.-ketoglutarate, succinyl-CoA, succinate, fumarate, malate, and oxaloacetate) is produced. In general terms, the culture media and/or culture conditions can be classified into one of two categories: those that promote cellular respiration and/or the production of biomass and those that reduce cellular respiration. Typically, culture media and/or culture conditions that promote cellular respiration are used in situations where rapid growth is needed, or where the organic product to be produced cannot be produced without cellular respiration. Such organic products can include, without limitation, Krebs cycle products. On the other hand, culture medium and/or culture conditions that reduce cellular respiration are used in situations where rapid growth is not needed or not desired, or where the organic product to be produced can be produced without cellular respiration. Such organic products include, without limitation, ***lactate***, acrylate, and xylose. (emphasis added)

Col. 20, lines 34-53:

Directed manipulation of culture conditions during a commercial production can be an important step in achieving optimal levels of a desired organic product as described herein. Typically, a yeast cell within the scope of the invention is grown under culture conditions that promote cellular respiration to produce a significant cell density. For example, yeast cells can be placed into a culture vessel, and given an abundance of glucose and oxygen. Typically, under conditions that promote cellular respiration, the doubling time for the microorganisms provided herein is less than about 10 hours (e.g., less than about 8, 5, or 3 hours). Once the cells reach a significant density, the culture conditions can be switched to conditions that reduce cellular respiration such that an organic product not requiring cellular respiration is produced. For example, the yeast cells can be transferred to a culture vessel and given an abundance of glucose, but no oxygen. In this case, directly manipulating the culture conditions such that they are switched from aerobic to anaerobic can produce optimal levels of a desired organic product.

Col. 23, lines 28-38:

Because energy cannot be created or destroyed, the cell requires an input of energy from the environment to maintain the order. Energy is generally supplied from the environment in the form of electromagnetic radiation or chemical energy. The energy obtained from the environment is harnessed by the cell used by one of two general biochemical mechanisms: substrate level phosphorylation and electron transport.

Generally, under anaerobic conditions, ATP (the "cellular currency" for energy) is produced by substrate level phosphorylation. In substrate level phosphorylation, energy is released from chemical bonds and is stored mainly in the form of ATP.

An example of substrate level formation is the conversion of glucose to pyruvate through glycolysis:

Glucose=2Pyruvate+2ATP+2H.sub.2

Pyruvate can be then be converted into lactic acid:

Pyruvate+2H.sub.2=Lactate

The net energy produced by the above transformation is equivalent to 2 ATP.

Col. 25, lines 1-4:

It is anticipated that in the later stages of a process, when high levels of metabolic products such as lactic acid are present, that the cell may require more metabolic energy to maintain function.

Col. 26, lines 15-54:

> In one embodiment, the present invention uses genetically modified yeast having a crabtree-negative phenotype in a train-type process that induces a "switch" in the metabolic pathway after a critical cell density has been reached and at which time it is desired to dramatically increase the specific productivity of the desired organic product. A typical method for inducing the metabolic pathway switch is by moving the biomass from a highly aerated vessel to a substantially anaerobic vessel, causing oxygen starvation. It is noted that a common carbohydrate (e.g., glucose or xylose) can be used as the carbon source during both the growth phase and the production phase. The use of a genetically modified yeast cell having a crabtree-negative phenotype can be critical to the success of this embodiment. In addition, the specific productivity of the desired organic product can be critical to success. The term "specific productivity" as used herein reflects the amount of product produced and is represented as the number of grams of organic product produced per gram of biomass (dry weight) per hour, i.e., g/(g*hour). Typically, the specific productivity for organic products such as lactate and acrylate is greater than about 0.1 g/(g*hour), for example, greater than about 0.2 g/(g*hour), or greater than about 0.5 g/(g*hour). *By providing a high specific productivity as described herein, the energy required for cell maintenance may be obtained via the fermentative product pathway under substantially anaerobic conditions*, rather than relying on aeration to generate high amounts of energy via the respiratory pathway. It is noted that substantially anaerobic vessels are aerated at a rate of less than about 0.1 VVM. Under certain production situations, no aeration will be used. In addition, the yield (i.e., g organic product/g carbon source consumed) in this embodiment typically is greater than about 70 wt %, and is produced without the addition of carbon sources such as ethanol and acetate. In some cases, in order to achieve the specific productivity required to generate the required energy for cell maintenance, it may be necessary to enhance the pathway from glucose to pyruvate in addition to providing the necessary enzymes to produce the desired product. (emphasis added)

From the foregoing passages, the person of ordinary skill in the art would understand Rajgarhia teaches a two-stage process for producing lactate in an engineered yeast. First, the practitioner would *grow* the engineered yeast under aerobic conditions (with air flow) to increase biomass; second, the practitioner would *switch* the engineered yeast to anaerobic conditions (with very little or no air flow) to *produce* lactate. The person of ordinary skill in the art would also understand that the higher the specific productivity, for example, the greater the amount of lactate produced per g biomass per hour, the more metabolic energy would be available to the

yeast, because along with each molecule of lactate produced are produced two molecules of ATP, the yeast cell's energy currency. The person of ordinary skill in the art would also understand that the more metabolic energy available, the better the yeast cell would function when large amounts of lactic acid have been accumulated late in the process.

In light of these passages, the person of ordinary skill in the art would be motivated to increase the specific productivity of lactate by an engineered yeast.

Further passages of Rajgarhia provide guidance on how to increase the specific productivity of lactate by an engineered yeast. At Example 15, col. 38, lines 16-44, Rajgarhia teaches:

> Large-Scale Production of Lactate
>
> Multiple variants of K. marxianus cells having reduced PDC activity are produced and isolated. Each variant is engineered to contain a different copy number of an exogenous nucleic acid molecule encoding a polypeptide having LDH activity. The LDH polypeptide is from multiple different sources. Such variant cells can have different specific productivity for lactic acid at 40.degree. C.
>
> Each variant is grown in a vessel under aerobic conditions with an air flow of 1.5 VVM and a dissolved oxygen content of 30% to reach a cell density of about 60 g/L, dry basis. Once the density is sufficient, the air flow is turned off, and the conditions within the vessel are switched to anaerobic conditions. No base is added. The variants with the highest specific productivity during the anaerobic phase can be found not only to produce lactic acid faster, but also to achieve a higher concentration at a lower pH, than the variants with lower specific productivity. Product yield on glucose during the production phase can exceed 90%.
>
> Certain variants are selected and subjected to the same culturing methods except that the air flow is reduced to 0.1 VVM, rather than being completely shut off. Under such conditions, the final pH within the vessel can be lower, and the lactate concentration can be higher than the conditions with no air flow. Product yield on glucose can be reduced but can remain at about 90%. When the test is repeated, but with an air flow of 0.5 VVM, the product yield on glucose can be reduced to less than 80%.

From this passage, the person of ordinary skill in the art would conclude that the specific productivity of the yeast variants is dependent on the copy number of the exogenous nucleic acid molecule encoding a polypeptide having LDH activity. He or she would readily expect that, *after switching from aerobic cell growth to anaerobic lactic acid production*, the greater the copy number of the nucleic acid molecule per cell, the greater the amount of the polypeptide having LDH activity per cell, and thus the greater specific productivity of lactic acid. The greater the specific productivity of lactic acid in the variant, the faster it will produce lactic acid, the greater the concentration of lactic acid, and the lower the pH of the medium.

The person of ordinary skill in the art could then routinely choose from among the variants, which differ only in the copy number of the exogenous nucleic acid molecule encoding a polypeptide having LDH activity, the one or ones that provide a particular combination of final lactate concentration and final lactate yield from glucose, depending on which combination of properties and production conditions are most desired. Generally speaking, the person of ordinary skill in the art can be presumed to want to maximize the specific productivity of lactic acid, the concentration of lactic acid produced, or the yield of lactic acid on glucose.

It must be borne in mind that this routine choice by a practitioner is not "selection on a parent yeast strain," as the term "selection" is used in the specification at, among other places, p. 11, line 18 to p. 12, line 25; p. 20, lines 1-8; p. 21, lines 10-14; and p. 25, line 13 to p. 27, line 6. Further, this routine choice is of a variant strain with a high specific productivity of lactic acid *after switching the strain to produce lactic acid*; the ability of the strain to *grow* at a lower pH than another strain is irrelevant.

If the person of ordinary skill in the art were then to consider modifications to Rajgarhia, he or she would be motivated to increase the specific productivity, produced concentration, or

yield of one or more of Rajgarhia's yeast variants. He or she would *lack* any motivation to increase the yeast variant's ability to *grow* at low pH. Rajgarhia teaches that when yeast variants are growing, they do *not* change the pH of their culture medium (Example 8, Figures 10-11). The person of ordinary skill in the art would expect this, because the yeast variants are *not* producing lactic acid during the growth phase. Rajgarhia teaches the adjustment of culture medium pH through the addition of mineral acids or bases (*ibid*). The person of ordinary skill in the art might therefore be motivated to adjust the pH of the growth phase of the yeast variants by the use of mineral acids or bases, in order to optimize the growth rate or final cell density of the yeast variant. Adjusting the pH of a medium is a simple, well-understood technique to the person of ordinary skill in the art.

Therefore, the person of ordinary skill in the art, having Rajgarhia before him or her, would simply choose a yeast variant with a high copy number of the exogenous nucleic acid molecule encoding a polypeptide having LDH activity, culture it in a medium with an appropriate pH during the growth phase, and then switch it to a production phase wherein its high copy number allows it to produce both more lactic acid and more ATP to survive at low pH.

If the person of ordinary skill in the art then happened upon Lee, he or she would note that Lee's selection process is performed on growing *Lactobacillus* (bacterial) cells to find *Lactobacillus* cells that simultaneously grow and produce lactic acid more rapidly than prior *Lactobacillus* cells.

The person of ordinary skill in the art would *lack* any motivation to apply the teachings of Lee to those of Rajgarhia for at least one or more of the following reasons:

1. Rajgarhia teaches the routine adjustment of the pH of the medium in the growth phase while leaving yeast variants unchanged, which is a far simpler than performing selection with

multiple additions of fresh medium and the risk of arriving at unproductive dead ends. (Lee, p. 6).

2. Rajgarhia is silent regarding the optimal pH for lactic acid production. However, whatever the optimal pH might be, the person of ordinary skill in the art could routinely adjust the pH of the culture medium to that optimal value at the same time he or she switches the yeast variant culture from the growth phase to the production phase. This is simpler than performing the longer, riskier process of Lee.

3. Lee teaches selection to find *Lactobacillus* mutants with improved *lactic acid production while growing cells* at low pH. This teaching is incompatible with Rajgarhia's plain teaching of growing yeast variant cells first and then producing lactic acid. If the person of ordinary skill in the art considered performing a selection like Lee's during the growth phase of Rajgarhia, he or she would conclude any resulting yeast variants would have no correlation with lactic acid production. If he or she considered performing a selection like Lee's during the production phase of Rajgarhia, he or she would conclude that, because the yeast variants of Rajgarhia do not grow during the production phase, there would be no mechanism to distinguish yeast variants by differential growth at low pH.

4. Rajgarhia teaches a straightforward way to increase both lactic acid production and ATP production at low pH during the production phase: increase the copy number of the exogenous nucleic acid molecule encoding a polypeptide having LDH activity. This directly combines two desired traits, high lactic acid productivity and metabolic viability. The selection process of Lee cannot directly combine these two traits and can lead to dead ends where viable, but non-lactic acid producing, *Lactobacillus* mutants are generated (p. 6).

5. Lee and Rajgarhia discuss different microorganisms from different biological kingdoms (prokaryotes and fungi). The person of ordinary skill in the art would therefore not be willing or able to apply the teachings of Lee to those of Rajgarhia.

6. As an example of the differences between the biological kingdoms, Rajgarhia plainly teaches that yeast are more acid tolerant than bacteria (col. 1, lines 25-46). As a result, the person of ordinary skill in the art would conclude that whatever mutations occurred in the *Lactobacillus* culture of Lee during Lee's selection process might correspond to wild type features of the yeast. In other words, Lee and Rajgarhia fail to give the person of ordinary skill in the art any reason to suspect that the yeast variants of Rajgarhia were not already at the maximum acid tolerance possible for yeast.

Therefore, the person of ordinary skill in the art would see no advantage, and several disadvantages, to modifying the teachings of Rajgarhia with those of Lee. This rejection should therefore be withdrawn.

The Examiner made a number of statements with which Applicants disagree. At p. 5 of the Detailed Action, he states "Selection of microbial cell that grow at most low pH is advantageous for increased production of lactic acid." This statement is unfounded on any fact in the record and cannot be considered common knowledge or well-known in the art for the reasons set forth above. As discussed above, Rajgarhia teaches growth and lactic acid production as two separate phases, and there is no evidence that growth at a low pH would later lead to increased production. Applicant therefore calls for the Examiner to support this statement by an affidavit. 37 CFR 1.104(d)(2).

The Examiner appears to have misunderstood "achieve a higher concentration [of lactic acid] at a lower pH" from Rajgarhia, Example 15, as meaning the lower the pH of the medium

prior to lactic acid production, the higher the concentration of lactic acid would be. Applicants submit, in light of the context of Rajgarhia, Example 15, that the quoted passage means that the higher the concentration of lactic acid, the lower the pH as a result of production.

The Examiner also alleged Rajgarhia, at col. 25, lines 20-50, teaches "yeast strain that produce lactic acid at lowest pH will produce most lactic acid and require least purification step." The cited passage makes no statement that yeast strains produce more lactic acid at lower pH. Regarding purification, it teaches "Specifically, the pH value of organic acids can precipitate out of solution when the pH of the broth is less than the pKa value for the organic acid. For example, the culture conditions while producing glutamic acid can be such that the pH is less than 2.19, which is the pKa value for glutamic acid." The pKa for lactic acid is 3.85; therefore, at any pH less than 3.85, and regardless of how much less, lactic acid would be expected to precipitate out of solution.

Even if Applicants do not explicitly dispute in this paper a statement made by the Examiner in the Detailed Action, Applicants do not necessarily acquiesce in that statement. The references are properly characterized above.

The Examiner also rejected claim 8 as allegedly being obvious over Rajgarhia and Lee, further in view of Hause, *et al.*, US 2003/0228671 ("Hause"). Applicants traverse this rejection.

The Examiner points to Hause as teaching the production of lactic acid by engineered yeast that do not produce glycerol. However, even if the person of ordinary skill in the art used the engineered yeast of Hause in the lactic acid production process of Rajgarhia, he or she would *lack* any motivation to perform any selection on that engineered yeast to produce a strain able to

grow at a lower pH than its parent strain for the reasons set forth above with reference to Rajgarhia and Lee. Therefore, this rejection should be withdrawn.

The Examiner also rejected claim 21 as allegedly being obvious over Rajgarhia and Lee, further in view of Rajgarhia, *et al.*, US 2004/0029256 ("Rajgarhia Published Application"). Applicants traverse this rejection.

The Examiner points to Rajgarhia Published Application as teaching a recombinant yeast strain expressing an exogenous LDH gene from *Lactobacillus plantarum*. However, even if the person of ordinary skill in the art used the recombinant yeast of Rajgarhia Published Application in the lactic acid production process of Rajgarhia, he or she would *lack* any motivation to perform any selection on that recombinant yeast to produce a strain able to grow at a lower pH than its parent strain for the reasons set forth above with reference to Rajgarhia and Lee. Therefore, this rejection should be withdrawn.

The Examiner also rejected claims 9-10 as allegedly being obvious over Rajgarhia and Lee, further in view of Porro, *et al.*, US 7,049,108 ("Porro"). Applicants traverse this rejection.

The Examiner points to Porro as teaching a recombinant *Saccharomyces cerevisiae* yeast strain expressing an exogenous LDH gene. However, even if the person of ordinary skill in the art used the recombinant *Saccharomyces cerevisiae* of Porro in the lactic acid production process of Rajgarhia, he or she would *lack* any motivation to perform any selection on that recombinant yeast to produce a strain able to grow at a lower pH than its parent strain for the reasons set forth above with reference to Rajgarhia and Lee. Therefore, this rejection should be withdrawn.

5. *Allowable subject matter*

The Examiner indicated claim 11 is allowable.

6. *Conclusion*

All pending claims are in condition for allowance. The Examiner is invited to contact the undersigned patent agent at (713) 934-4065 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

WILLIAMS, MORGAN & AMERSON, P.C.
CUSTOMER NO. 79138

June 3, 2009

/Raymund F. Eich/
Raymund F. Eich, Ph.D.
Reg. No. 42,508

10333 Richmond, Suite 1100
Houston, Texas 77042
(713) 934-4065
(713) 934-7011 (fax)

AGENT FOR APPLICANTS